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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/87, A61K 48/00, 47/48, 31/70, C07K 14/82

A1

(11) International Publication Number: **WO 96/39531**

(43) International Publication Date: 12 December 1996 (12.12.96)

(21) International Application Number: PCT/US96/08827

(22) International Filing Date: 5 June 1996 (05.06.96)

(30) Priority Data:
08/471,642 6 June 1995 (06.06.95) US

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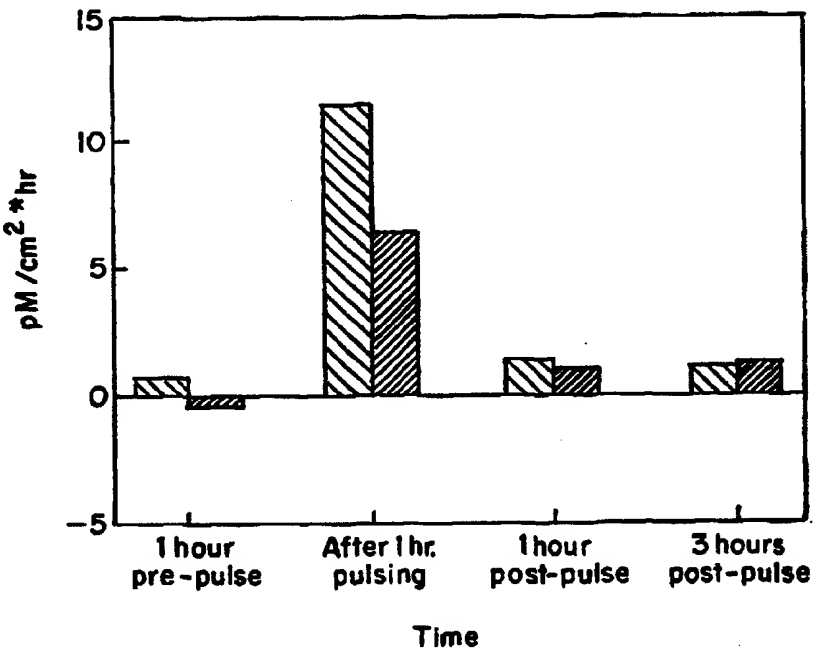
(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DELIVERY OF NUCLEOTIDES INTO ORGANISMS BY ELECTROPORATION



(57) Abstract

A method for delivering a nucleotide into an organism includes applying a composition which includes a nucleotide component to epidermis of the organism. The epidermis is electroporated, whereby at least a portion of the composition enters or passes across the epidermis, thereby delivering the nucleotide into the organism. An example of a suitable nucleotide which can be delivered by the method of the invention includes antisense oligodeoxynucleotides for treatment of melanomas.

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DELIVERY OF NUCLEOTIDES INTO ORGANISMS BY ELECTROPORATION

Background of the Invention

Many drugs and chemical agents are known to be effective in treatment of diseases. However, such agents also often have deleterious side effects when introduced into the organism in sufficient dosage to treat the targeted tissue.

One attempt to selectively treat diseased tissue is development of chemical agents which selectively affect only the diseased tissue. However, such chemical agents typically are only partially selective for the diseased tissue and often have a deleterious effect on healthy cells. Another attempt to selectively treat diseased tissue is to inject the chemical agent directly into the diseased tissue mass. However, the effect of chemical agents on diseased tissue is often dependent upon delivery of the chemical agent across cell membranes of the cells in the tissue mass as opposed to simply injecting the chemical agent into the tissue. Further, chemical agents which are injected into diseased tissue typically enter the bloodstream and are transported away from the targeted tissue mass before they have a significant therapeutic effect on the tissue mass into which they were injected.

In other specific applications of drugs, such as gene therapy, nucleotides are delivered to alter the behavior of cells. For example, antisense oligonucleotide therapy is based on the premise that transcription of a gene can be blocked when a cell is exposed to an antisense oligonucleotide corresponding to that gene. This gene can code, and thereby lead to the production of a protein which causes cancer (an oncogene) or a protein necessary for viral replication. Antisense oligonucleotide therapy for

several forms of cancer (e.g., lymphomas and melanomas) and viruses (e.g., human t-cell leukemia virus (HTLV-I) and human immunodeficiency virus (HIV)) have been successfully performed in vitro and in mice.

However, there are many problems that are often associated with treatment of cells by conventional methods, such as intravenous injection. For example, the cells of melanoma tumors are typically difficult to target by injection techniques because they are in the form of relatively thin tissue. Further, injections can traumatize tissue, thereby possibly spreading potentially malignant growths. In addition, some therapeutic nucleotide compositions, such as those that include antisense oligonucleotides, are very expensive and, consequently, require very localized application. Also, use of some types of intravenous injection, such as intravenous infusion pumps, can be difficult to control and can promote infection. This complication is especially significant for patients afflicted with immunocompromising illnesses (e.g., leukemias and HIV infection).

Therefore, a need exists for a new method for delivering nucleotides into organisms.

Summary of the Invention

The present invention relates to a new method for delivering a nucleotide into an organism.

The method includes applying a composition having a nucleotide component to epidermis of the organism. The epidermis is electroporated, whereby at least a portion of the composition enters or passes across the epidermis, thereby delivering the nucleotide into the organism.

The method of the invention is advantageous in several respects. First, the method enables topical treatment of skin lesions having a genetic component, such as melanoma tumors. This treatment is not invasive and delivery of

nucleotides can be localized to the site of the lesion. Further, the amount of nucleotide necessary to treat a particular lesion is significantly reduced by localized application of the nucleotide, thereby substantially diminishing the cost of treatment. In addition, mechanical trauma, such as that caused by subcutaneous injections, is avoided by electroporation. Further, risk associated with disrupting cancer cells, such that they are moved to another location, thereby spreading the cancer, is lessened. With regard to systemic illnesses, delivery of nucleotides, such as antisense oligonucleotides, by the method of the invention can be controlled to ensure shut-off of the targeted gene over an extended period of time. Also, the likelihood of infection associated with delivery by intravenous injection is avoided by electroporation.

Brief Description of the Drawings

Figure 1 shows a bar graph of fluorescein-labelled oligodeoxynucleotide transport through skin in vitro before, during, and after electroporation.

Figure 2 shows a bar graph of fluorescein-labelled oligodeoxynucleotide transport through skin in vitro as a function of transdermal voltage.

Figure 3 is a bar graph of fluorescein-labelled oligodeoxynucleotide transport through skin in vitro, as a function the duration of a pulse-time constant.

Figure 4 is a photograph showing distribution of fluorescein-labelled oligodeoxynucleotide in skin in vitro.

Detailed Description of the Invention

The features and other details of the method of the invention will now be more particularly described and pointed out in the claims. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The

principle features of this invention can be employed in various embodiments without departing from the scope of the invention.

Generally, electroporation is a method of increasing the permeability of tissue and cell membranes. The increased permeability allows transport, or migration, of chemical agents through the tissue or across cell membranes into cells. For example, electroporation can include applying a voltage across tissue in vitro to cause the permeability of the tissue and cell membranes of cells in the tissue to increase. If the tissue is in the presence of a suitable chemical agent, the chemical agent can then migrate across the tissue or into cells of the tissue. Electroporation has also been used to deliver drugs to tissue, in vivo, by applying electrodes to the surface of an organism and applying a voltage between the electrodes which exposes the tissue to an electric field. The tissue thereby becomes electroporated and allows delivery of a chemical agent, such as a drug, which has been applied either topically to the organism or injected into the blood stream of the organism, across the electroporated tissue and into cells of the electroporated tissue.

"Electroporation," as that term is used herein, means increased permeability, of a cell membrane and/or at least a portion of cells of a targeted tissue, to a chemical agent, wherein the increased permeability is caused by application of voltage across the cell or at least a portion of the tissue. The chemical agent can thereby migrate into or across the electroporated tissue, and/or across the cell membrane and into the cell. Electroporation can include, for example, injuring at least a portion of the cells of targeted tissue to thereby cause the permeability of the tissue to significantly increase.

Electric pulses are generated to cause electroporation by the method of the invention. Examples of suitable

apparatus for conducting electroporation according to the method of the invention are set forth in U.S. 5,019,034, issued to Weaver et al. on May 28, 1991, and U.S. 5,389,069, and U.S. 5,389,069, issued to Weaver on February 14, 1996. Typically, the electrical pulses are exponential pulses having a time constant in the range of between about 0.1 and about three milliseconds, or between about 0.1 and about 0.3 seconds, and an amplitude in the range of between about fifty and about one thousand volts. However, the pulse configuration can also be square, bipolar, etc. Generally, the number of pulses sufficient to cause electroporation is in the range of between about one and about ten, wherein the interval between pulses is in the range of between about 0.01 second and one minute. Usually, the largest increase in permeability caused by electroporation occurs as a consequence of applying the first pulse.

The method of the invention includes delivering a nucleotide into an organism. In particular, the method includes applying a composition which includes a nucleotide component to epidermis of the organism and then electroporating the epidermis, whereby at least a portion of the composition enters or passes across the epidermis, thereby delivering the nucleotide into the organism.

Examples of suitable nucleotides include polynucleotides, oligonucleotides, deoxyribonucleotides and oligodeoxyribonucleotides. Other specific examples of suitable nucleotides include deoxynucleotides, such as oligodeoxynucleotides. Examples of suitable oligodeoxynucleotides include antisense oligodeoxynucleotides, such as an antisense oligodeoxynucleotide which corresponds to at least a portion of a codon sequence in a c-myc gene. For example, the antisense oligodeoxynucleotide can be a 15-mer having a nucleotide sequence of 5'-AACGTTGAGGGGCAT-3' (SEQ. ID NO:

1). The c-myc antisense oligodeoxynucleotide can correspond to codons 1-5 of the c-myc gene.

Alternatively, the antisense oligodeoxynucleotide can correspond to at least a portion of a codon sequence in a c-myb gene. For example, the antisense oligodeoxynucleotide can be a 24-mer having a nucleotide sequence of 5'-TATGCTGTGCCGGGGTCTTCGGGC-3' (SEQ. ID NO: 2). The c-myb antisense oligodeoxynucleotide can correspond to codons 2-9 of the c-myc gene.

Examples of other suitable components of the composition applied to the epidermis of the organism include, for example, a reducing agent, such as a charged reducing agent, that disrupts crosslinked keratin within keratinocytes of the epidermis.

Optionally, the epidermis can be pretreated by application of a suitable polyanion. Examples of suitable polyanions to inhibit non-specific deoxyribonucleic acid (DNA) binding and, thus, diminish the amount of DNA which, following electroporation, might be trapped in the epidermis. Examples of suitable polyanions include bovine serum albumin and ficol.

In another option, the epidermis can be treated by application of a proteinase, such as keratinase, papain, or reducing agents or compounds, to overcome hindrance of DNA transport during electroporation that might be caused by the dense keratin matrix of the epidermis.

A sufficient voltage is applied to the portion of the epidermis to which the composition was applied to cause a fraction of the area of that portion of the epidermis to become electroporated. Typically, the epidermis is electroporated by achieving a transdermal voltage of at least about 80 volts. In one embodiment, the transdermal voltage is applied as a series of pulses. In a specific embodiment, the pulsed-applied transdermal voltage has an

average duration in a range of between about 1.1 milliseconds and about 2.2 milliseconds per pulse.

The electroporated portion of the epidermis can include the stratum corneum. As a consequence of electroporation, the applied composition at the epidermis enters the epidermis. In one embodiment, the composition can pass across the epidermis from a first side of the epidermis to a second, basal side of the epidermis.

Optionally, electroporation of the skin can be conducted in conjunction with an additional electrical protocol, such as iontophoresis. The additional application of an electrical field can be conducted prior to, during, or after electroporation of the epidermis.

In another embodiment, the composition applied to the epidermis can be a vaccine, such as a vaccine that includes a plasmid deoxyribonucleic acid component. Alternatively, the composition can include a deoxyribonucleotide analog, such as azidodeoxythymidine, dideoxyinosine, dideoxycytosine, gancyclovir, acyclovir, vidarabine, ribavirin, etc.

In other embodiments, the composition can include a label, such as a radioactive label. Alternatively, the composition can include a photoactive modification, such as Psoralin C2.

In still another embodiment, the composition can include a phosphoramidate linkage, such as butylamidate, piperazidate, and morpholidate. Alternatively, the composition can include a phosphothiolate linkage or ribonucleic acid. These linkages decrease the susceptibility of the oligonucleotides and polynucleotides to degradation.

Optionally, the electrical resistance or impedance of the epidermis can be measured during electroporation to thereby monitor the amount of electroporation that has occurred.

In another embodiment, a composition which includes a nucleotide can be applied to a tissue within an organism. The tissue is electroporated, whereby at least a portion of the composition enters or passes across the tissue, thereby delivering the nucleotide into the tissue. Suitable apparatus for conducting this embodiment of the method of the invention are disclosed in U.S. 5,389,069, the teachings of which are incorporated herein by reference in their entirety.

The invention will now be further and specifically described by the following examples. All parts and percentages are by weight unless otherwise specified.

Exemplification

MATERIALS AND METHODS

Fluorescein Labeled Oligonucleotide (ODN) Synthesis and Purification

1 mMole of each ODN was synthesized fully phosphorothioated on an Applied Byosystems 380B DNA Synthesizer. (G. Zon, W.J. Steck, in "Oligonucleotides and Analogues: A Practical Approach," F. Eckstein, Eds. (Oxford Univ. Press, Oxford, 1991), pp. 87-108.) The use of phosphorothioate linkages has been shown to greatly decrease nuclease digestion. A fluorescein CPG support (Glen Research, Sterling, VA) was used so that the fluorescein derivative was directly incorporated into the backbone of the ODNs at the 3′ end from the start of the synthesis. ODNs were ethanol precipitated, washed in 70% ethanol, detritylated and further purified using NENSORB PREP cartridges (E.I. duPont de Nemours & Co., NEN Products, Boston, MA). Purity (i.e., no smaller ODNs or free fluorescein) was confirmed by denaturing polyacrylamide electrophoresis prior to and after electroporation. The sequence for the antisense 15-mer of

c-myc and the 24-mer of c-myb were 5'-AACGTTGAGGGGCAT-3' (SEQ. ID NO. 1) and 5'-TATGCTGTGCCGGGGTCTTCGGGC-3' (SEQ. ID NO. 2), respectively. The c-myc antisense ODN was 4.8kDa and corresponded to codons 1-5 of the c-myc gene; the c-myb antisense ODN was 7.0kDa and corresponded to codons 2-9 of the c-myb gene.

Skin Preparation

Heat-stripped stratum corneum was used in all experiments (area exposed to the electrical field = 0.7 cm^2). (M.R. Prausnitz, et al., *Proceed. Intern. Symp. Control. Rel. Bioact. Mater.*, 20, 95-96 (1993)). The skin was obtained from either the abdomen, arm, or back of adult human cadavers. Prior to heat-stripping the skin was stored at -70°C for one to six months. After heat-stripping, the skin was stored at 4°C in a 95% humidity environment.

Pulse Application and Electrical Measurements

High-voltage pulses were delivered using an exponential pulser (Electroporation System 600, BTX Industries, San Diego, CA) modified for automated control. The pulse time constants t_{pulse} was varied from 1.0 to 2.2 ms (using 50Q to 720W internal shunt resistance) with 0.1 ms variation during an experiment. A side-by-side permeation chamber was used. Two pairs of Ag/AgCl electrodes constructed from 16-gauge silver wire were used for both the application and sensing of voltage. The donor compartment was at the negative and the receptor compartment at the positive side of the pulser. This forward direction polarity provided an electrophoretic driving force through the skin for the negatively charged fluorescent molecules. A voltage divider effect involving the bathing solution, electrodes, and skin resulted in the

transdermal voltage being much less than the pulsing voltage. Voltage traces were acquired and stored on a Hewlett-Packard 54601 digital oscilloscope.

Image Acquisition

A biocular fluorescence microscope (Olympus BH-2) was used, together with an Olympus OM2 camera. Shutter times were between 0.25 and 3 seconds.

Fluorescent Molecule Transport Across the Stratum Corneum

The concentration of ODN in the donor compartment was 25 mm. The fluorescence of the receptor compartment solution was measured using a spectrofluorimeter (Fluorolog 2, Model F112AI, SPEX Industries, Edison, NJ). To visualize sites of local transport, a 2% agarose solution in phosphate-buffered saline at 40°C was added to the receptor compartment after one hour of pulsing, and allowed to gel for five minutes. A flow-through water jacket (23±3°C) in the side-by-side apparatus was connected during these procedures. (M.R. Prausnitz, V.G. Bose, R. Langer, J.C. Weaver, *Proc. Natl. Acad. Sci. USA*, 90, 10504-10508 (1993)). Pulsing at approximately 80V transdermal was carried out for three minutes, and then the agarose was viewed under a long-wave UV lamp for fluorescence visualization.

Imaging of Small Ion Transport Regions

To examine regions which retained transport ability up to several hours after pulsing, we removed the skin from the side-by-side apparatus and then placed it on a polished silver surface with the stratum corneum side touching the surface. Iontophoresis (1mA for 30 seconds) was performed, and sites of ion deposition onto the silver were noted, as can be seen in Figure 4.

Results

Figure 1 is a bar graph of fluorescein ODN transport through the skin. Total molecular flux of fluorescein-labeled c-myc and c-myb ODNs for one hour prior to pulsing, during one hour of exponential (t_{pulse}=1.1 ms) pulsing at 80V (U_{skin}) every five seconds, for one hour after pulsing, and for three hours further after pulsing. The flux standard deviation during pulsing is 3.5 for c-myc and 2.1 for c-myb. The errors in the other flux measurements were greater than 1.5 pM/cm^2 * hr; therefore, the measured value corresponding to flux before and after pulsing was less than the statistical error inherent in the measurement.

Figure 1 shows that there was no significant flux of either of the fluorescein ODNs either before or after pulsing; whereas, significant transport occurs during pulsing. Statistically significant flux values for passive flux after pulsing were not obtained after even the highest voltage.

Figure 2 is a bar graph of fluorescein ODN transport through skin as a function of transdermal voltage. The conditions are the same as those set forth for the example represented by Figure 1. Figure 2 shows a sharp increase in transport of both fluorescein ODNs in raising the transdermal pulse voltage from 69V to 80V. The transport at 69V is less than the error associated with the measurement, but increasing the voltage from 80V to 139V increases transport about one standard of deviation for fluorescein c-myc. The amount of flux appeared to plateau above 80V.

Figure 3 is a bar graph of fluorescein ODN transport through skin as a function of pulse time constant. The conditions are the same as those set forth for the example represented by Figure 1. Figure 3 shows that transport of both c-myc and c-myb fluorescein ODNs increases with

increasing exponential pulse time constant. Under almost all conditions the flux of the c-myc ODN is greater than that of the c-myb ODN, although the difference is typically on the order of one standard of deviation.

Figure 4 is an image based on fluorescein ODN in the skin and ion transport through the skin. After pulsing at 92V (U_{skin}), the skin was placed between an agarose gel disk containing PBS and a polished silver electrode (stratum corneum touching this electrode). Iontophoresis was then carried out by maintaining a constant current (1mA) through the skin for 30 seconds with the polished silver electrode serving as the anode and the agarose disk attached to a cathode. Anions (mainly, chloride) deposited onto the anode after having traversed the skin creating the dark areas. The images are of the skin and underlying silver electrode after the agarose disk had been removed. Areas of localized fluorescein ODN staining were bright. Illumination with white light and blue light (480 nm cutoff). Comparing the bright and dark it was seen that the areas of fluorescent molecule and current flux roughly coincided, with the latter having been more diffuse.

As shown in Figure 4, fluorescence microscopy revealed the fluorescein ODNs were localized to areas 26±8 mm in diameter in the skin. Further, fluorescent ODNs were observed to penetrate all of the keratinocyte layers of the stratum corneum, and no structural damage was observed at 400x within these areas. Fluorescent ODNs were always located in the area of the heat-stripped skin corresponding to the dermal papillae (troughs of the epidermis) and no localized regions of staining appears in the Rete pegs (crests of the epidermis). 135±24 localized centers of staining per cm^2 skin were observed after pulsing at 80V with a time constant of 1.1 ms for one hour every 5 seconds. Significantly, there was little or no

fluorescence in the skin appendages (hair follicles and sweat ducts).

A relationship between localized skin fluorescence and actual transport was established by retaining the fluorescent molecules crossing the stratum corneum within a 2% agarose gel in the receptor compartment of the side-by-side apparatus. The areas of fluorescence in the gel were much broader than those in the skin, presumably due to lateral diffusion within the gel. A fluorescent region in the gel always corresponded to a fluorescent region in the skin stained with fluorescent DNA corresponded to a fluorescent area in the gel. No staining of the agarose occurred at areas corresponding to sweat ducts and hair follicles, indicating that relatively few molecules had been transported through these structures.

The existence of ionic transport pathways which persist after pulsing was observed, as also shown in Figure 4. Generally the areas with fluorescent molecule and current flux coincided, with the latter being larger.

Discussion

Fluorescein-labeled ODNs were transported through skin in vitro during high-voltage pulsing. Transport increased markedly for pulsing with the transdermal voltage more than about 70V, but quickly plateaued, an effect which had also been observed for calcein uptake by red blood cell ghosts and yeasts. (M.R. Prausnitz, et al., *Biophys. J.*, 65, 414-422 (1993)) (E.A. Gift, J.C. Weaver, *Biochim. Biophys. Acta*, 1234:52-62 (1995). In contrast, transport increased with increasing t_{pulse} from 1.1 to 2.2 ms. This result and the observation that passive flux was negligible suggested that electrophoresis during the electrical pulses was important.

The observation of fluorescence in all layers of the stratum corneum by stereomicroscopy and the retention of fluorescence in the agarose gel were direct evidence for localized transport regions (LTRs) across the skin. Moreover, these results were in accord with what has been seen for the transport of the smaller, negatively-charged molecules calcein and sulforhodaine across skin.

Transport during high-voltage pulsing occurred primarily in LTRs which did not correspond to appendages. Also, in the experiments with AgCl deposition during post-pulse iontophoresis, the deposition corresponding to the localized ODN fluorescence in the skin was, in general, greater (i.e., areas were darker) than the deposition behind the appendages. Similar results were seen for calcein and sulforhodamine for related pulsing protocols.

The fractional area of all the LTRs for ODN transport was 8×10^{-4} (after 720 pulses at 135V); this value was much less than that observed for calcein 8×10^{-2} (after only 40 pulses ranging from 80V to 158V). Both of these values were significantly more than the fractional area $F_w \approx 5 \times 10^{-5}$ that was estimated to actually participate as transporting aqueous pathways. Lateral transport of the fluorescent molecules could explain the difference between these observed and theoretical values. Moreover, the ODNs may have spread less laterally due to their relatively large size. Further, only approximately one in twenty DNA-stained regions actually transported; therefore, the net F_w is approximately 2×10^{-5}. Over 100 ng/cm^2-hr of DNA were transported through the human epidermis.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: Delivery of Nucleotides into Organisms by Electroporation

(iii) NUMBER OF SEQUENCES: 2

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/471,642
(B) FILING DATE: June 6, 1995

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AACGTTGAGG GGCAT 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TATGCTGTGC CGGGGTCTTC GGGC 24

CLAIMS

1. A method for delivering a nucleotide into an organism, comprising:
   a) applying a composition which includes a nucleotide component to epidermis of the organism; and
   b) electroporating the epidermis, whereby at least a portion of the composition enters or passes across the epidermis, thereby delivering the nucleotide into the organism.

2. The method of Claim 1 wherein the nucleotide is a polynucleotide.

3. The method of Claim 1 wherein the nucleotide is an oligonucleotide.

4. The method of Claim 1 wherein the nucleotide is a deoxyribonucleotide.

5. The method of Claim 4 wherein the deoxynucleotide is an oligodeoxyribonucleotide.

6. The method of Claim 1 wherein the nucleotide is a deoxynucleotide.

7. The method of Claim 6 wherein the deoxynucleotide is an oligodeoxynucleotide.

8. The method of Claim 7 wherein the oligodeoxynucleotide is an antisense oligodeoxynucleotide.

9. The method of Claim 8 wherein the antisense oligodeoxynucleotide corresponds to at least a portion of a codon sequence in a c-myc gene.

10. The method of Claim 9 wherein the antisense oligodeoxynucleotide is a 15-mer having a nucleotide sequence of 5'-AACGTTGAGGGGCAT-3' (SEQ. ID NO. 1).

11. The method of Claim 10 wherein the c-myc antisense oligodeoxynucleotide corresponds to codons 1-5 of the c-myc gene.

12. The method of Claim 8 wherein the antisense oligodeoxynucleotide corresponds to at least a portion of a codon sequence in a c-myb gene.

13. The method of Claim 12 wherein the antisense oligodeoxynucleotide is a 24-mer having a nucleotide sequence of 5'-TATGCTGTGCCGGGGTCTTCGGGC-3' (SEQ. ID NO. 2).

14. The method of Claim 13 wherein the c-myb antisense oligodeoxynucleotide corresponds to codons 2-9 of the c-myc gene.

15. The method of Claim 1 wherein the epidermis is electroporated by applying a transdermal voltage of at least about 80 volts.

16. The method of Claim 15 wherein the transdermal voltage is achieved by applying a series of pulses.

17. The method of Claim 16 wherein the pulsed applied transdermal voltage has an average duration in a range

of between about 0.5 milliseconds and about 50 milliseconds per pulse.

18. The method of Claim 1 wherein the electroporated epidermis is stratum corneum.

19. The method of Claim 1 wherein at least a portion of the composition enters the epidermis.

20. The method of Claim 1 wherein the composition passes across the epidermis from a first side of said epidermis to a second side of said epidermis.

21. The method of Claim 1 wherein said composition includes a vaccine.

22. The method of Claim 21 wherein said vaccine includes a plasmid deoxyribonucleic acid component.

23. The method of Claim 1 further including the step of applying a polyanion to the epidermis prior to electroporating said epidermis, thereby inhibiting non-specific DNA binding.

24. The method of Claim 23 wherein said polyanion includes an albumin.

25. The method of Claim 24 wherein said albumin includes bovine serum albumin.

26. The method of Claim 23 wherein said polyanion includes ficol.

27. The method of Claim 1 wherein the composition includes a deoxyribonucleotide analog.

28. The method of Claim 27 wherein said deoxyribonucleotide includes azidodeoxythymidine.

29. The method of Claim 27 wherein said deoxyribonucleotide includes dideoxyinosine.

30. The method of Claim 27 wherein said deoxyribonucleotide includes dideoxycytosine.

31. The method of Claim 27 wherein said deoxyribonucleotide includes gancyclovir.

32. The method of Claim 27 wherein said deoxyribonucleotide includes acyclovir vidarabine.

33. The method of Claim 27 wherein said deoxyribonucleotide includes acyclovir.

34. The method of Claim 27 wherein said deoxyribonucleotide includes ribavirin.

35. The method of Claim 1 wherein the composition includes a label attached to said nucleotide component.

36. The method of Claim 35 wherein said label is a radioactive label.

37. The method of Claim 1 wherein the composition includes a Psoralin C2.

38. The method of Claim 1 wherein the composition includes a phosphoramidate linkage.

39. The method of Claim 38 wherein said phosphoramidate linkage includes butylamidate.

40. The method of Claim 38 wherein said phosphoramidate linkage includes piperazidate.

41. The method of Claim 38 wherein said phosphoramidate linkage includes morpholidate.

42. The method of Claim 1 wherein the composition includes a phosphothioate linkage.

43. The method of Claim 1 wherein the composition includes ribonucleic acid.

44. The method of Claim 1 further including the step of applying a proteinase to said epidermis prior to electroporating said epidermis.

45. The method of Claim 44 wherein said proteinase includes keratinase.

46. The method of Claim 44 wherein said proteinase includes papain.

47. The method of Claim 1 wherein the applied composition further includes a reducing compound that causes disruption of crosslinked keratin within keratinocytes of said epidermis.

48. The method of Claim 47 wherein the reducing compound of said applied composition is a charged reducing compound.

49. The method of Claim 1, further including the step of measuring electrical resistance across the epidermis during electroporation, whereby electroporation of said epidermis can be monitored.

50. The method of Claim 1, further including the step of measuring electrical impedance across the epidermis during electroporation, whereby electroporation of said epidermis can be monitored.

51. A method for delivering a nucleotide into a tissue of an organism, comprising:

a) applying a composition which includes a nucleotide component to the tissue of the organism; and

b) electroporating the tissue, whereby at least a portion of the composition enters or passes across the tissue, thereby delivering the nucleotide into the tissue.

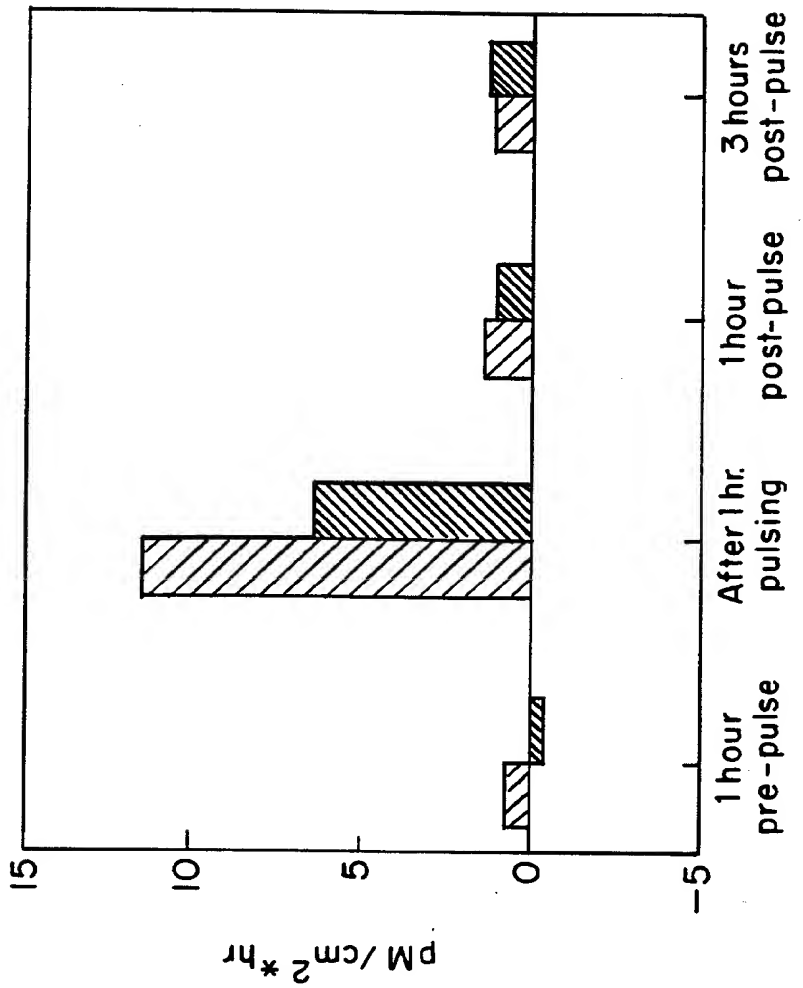
pM /cm2 *hr
15
10
5
0
-5
1 hour pre-pulse
After 1 hr. pulsing
1 hour post-pulse
3 hours post-pulse
Time

FIG. 1

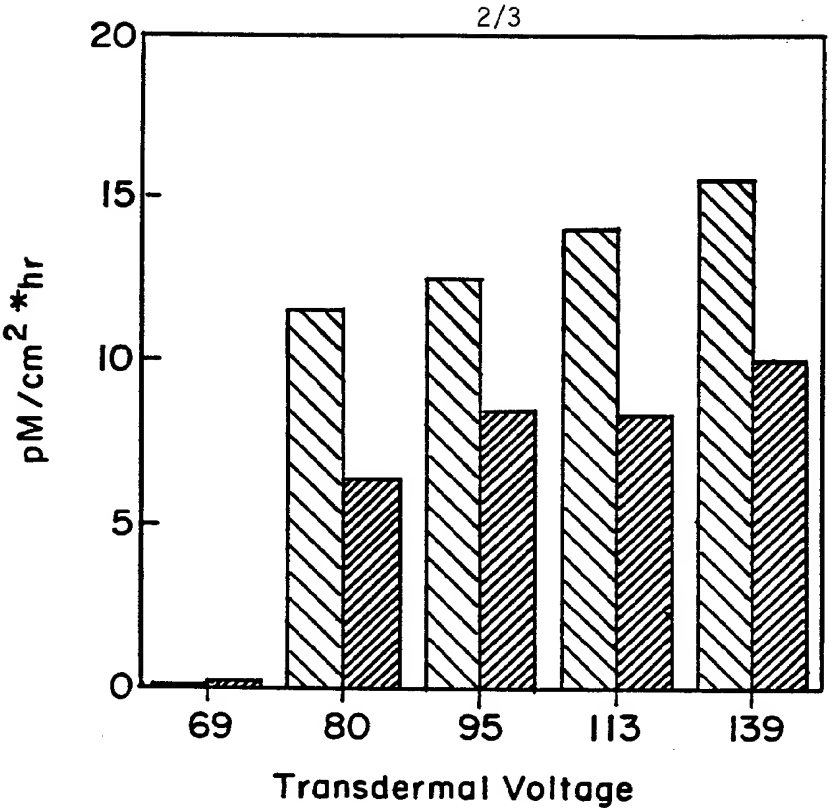
20
15
10
5
0
pM/cm2*hr
69
80
95
113
139
Transdermal Voltage

FIG. 2

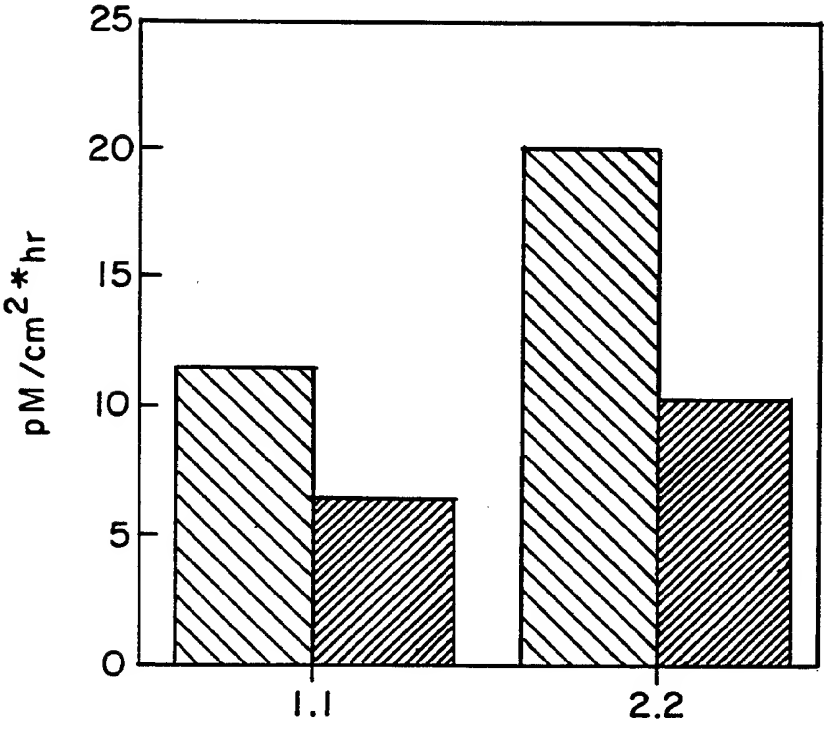
25
20
15
10
5
0
pM/cm2*hr
1.1
2.2
Time Constant (ms)

FIG. 3

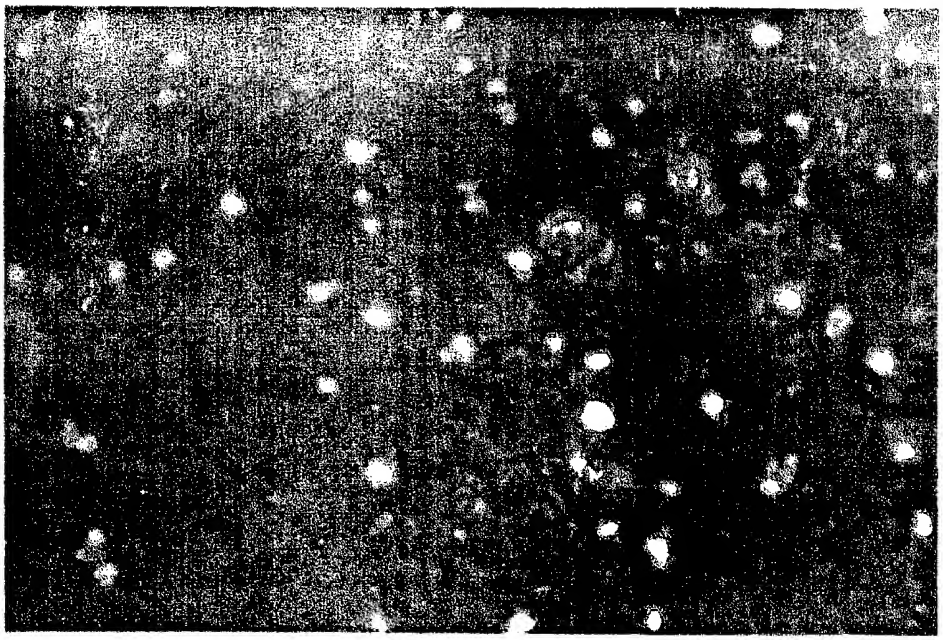

FIG.4

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/08827

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/87 A61K48/00 A61K47/48 A61K31/70 C07K14/82

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 389 069 (WEAVER JAMES C) 14 February 1995 cited in the application see the whole document ---	1,2,4, 16,17, 21,51
X	US,A,5 019 034 (WEAVER JAMES C ET AL) 28 May 1991 cited in the application see the whole document ---	1,15-20, 49,51
X	US,A,5 137 817 (BUSTA HEINZ H ET AL) 11 August 1992 see column 4, line 19 - line 37 see column 11, line 32 - line 42; claims 1-13; figures 4F,4G,8A --- -/--	1,2,4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 October 1996	18. 10. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chambonnet, F

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 90, no. 22, November 1993, WASHINGTON US, pages 10504-10508, XP000601520 PRAUSNITZ, M.R. ET AL.: "Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery"	1,18-20, 49,51
Y	see the whole document	1,3,6-12
P,X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 212, no. 2, 17 July 1995, ORLANDO, FL US, pages 286-292, XP000601613 ZEWERT, T.E. ET AL.: "Transdermal transport of DNA antisense oligonucleotides by electroporation"	1-18,51
P,Y	see the whole document	4,5, 13-20, 49,51
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3

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 96/08827

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US-A-5389069	14-02-95	US-A-	5019034	28-05-91
		US-A-	5547467	20-08-96
		AT-T-	131081	15-12-95
		CA-A-	1326268	18-01-94
		DE-D-	68925030	18-01-96
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		EP-A-	0398960	28-11-90
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		DE-D-	68925030	18-01-96
		DE-T-	68925030	25-07-96
		EP-A-	0398960	28-11-90
		JP-T-	3502416	06-06-91
		WO-A-	8906555	27-07-89
US-A-5137817	11-08-92	NONE		